

Marked-up Specification

In the specification:

Please delete pages 8-9 and insert the following:

-- Materials and Methods

The multiplex PCR sequencing method is applied for ascertaining the total polymorphic spectrum of the beta2 receptor gene. To this end, the overall promoter region so far and the coding region are subdivided into eight fragments and amplified by means of PCR (see Fig. 1). These PCR fragments were pooled and sequenced simultaneously. The fragments of the termination reactions were separated on a separate gel and transferred to a nylon membrane by means of direct transfer electrophoresis (DTE). The individual sequence leaders were successively decoded by successively hybridizing with specific oligonucleotides.

The specific conditions for the amplification were as follows:

Forward primer ADRBR-F1 with the sequence

5' - TATTGCCAGGATCTTGCTTCTAT-3' (SEQ ID No. 8) and backward

primer ADRBR-R1 with the sequence

5' - TAACATTAAGAACATTTGAAGC -3' (SEQ ID No. 9) were used for

fragment I. Fragment II was amplified by means of the two

primers ADRBR-F2 5' - GCATACCCCGCTCCAGATAAA -3' (SEQ ID No. 10)

and ADRBR-R2 5' - GCACGCACATACAGGCACAAATAC -3' (SEQ ID No. 11).

For fragment III it were two primers

ADRBR-F3: 5' - GGCGCGTTCTGTGTTGG -3' (SEQ ID No. 12) and

ADRBR-R3: 5' - AGTGCCTCTGCCGTTATGTG -3' (SEQ ID No. 13). For fragment VIII the two primers

ADRBR-F8: 5' - GGTACTGTGCCTAGCGATAAC -3' (SEQ ID No. 14) and

ADRBR-R8: 5' - TAAAATACCCCGTGTGAGCAAATAAGAG -3' (SEQ ID No. 15)

were used. The reactions conditions for these four fragments were as follows: 10 x PCR buffer (100 nM Tris HCl, 15 mM MgCl₂ x 6H₂O, 500 mM KCl, pH 8.3), dNTP 2 mM, 30 μM primer F, 30 μM primer R, 50 ng of genomic DNA and 5 U of a Taq DNA polymerase. All three fragments were amplified with the following temperature profile: 94°C 4 min; 35 cycles: 94°C 30 sec., 60°C 30 sec., 72°C 1 min. and finally 72°C 10 min.

Fragment IV was amplified with the aid of the two primers

ADRBR-F4: 5' - GGGGAGGGAAAGGGGAGGAG -3' (SEQ ID No. 16) and

ADRBR-R4: 5' - CTGCCAGGCCATGACCAGAT -3' (SEQ ID No. 17). For fragment VII the primers

ADRBR-F7: 5' - CTGGCTGCCCTCTTCATCGTT -3' (SEQ ID No. 18) and

ADRBR-R7: 5' - TACCCTAAGTTAAATAGTCTGTT -3' (SEQ ID No. 19) were used. The conditions for these two PCR reactions were as follows: 10 x PCR buffer (160 mM (NH₄)₂SO₄, 0.1% of Tween-20, 500 mM KOH, pH), dNTP 2 mM, 30 μM primer F, 30 μM primer R, 50 nG of genomic DNA and 4 U of a mixture of Taq DNA polymerase and

a thermostable inorganic pyrophosphatase of *thermos thermophilus*. Both fragments were amplified with the following temperature profile: 94°C 4 min.; 35 cycles: 94°C 30 sec., 66°C [fragment IV] or 60°C [fragment VII] 30 sec., 72°C 1 min. and finally 72°C 10 min.

Fragment V was amplified by means of the two primers

ADRBR-F5: 5'- ATGCCCGGACCGAC -3' (SEQ ID No. 20) and

ADRBR-R5: 5'- GTAGAAGGACACGATGGA -3' (SEQ ID No. 21), fragment VI was amplified with the two primers

ADRBR-R6: 5'- GCTACTTGCCATTACTTCACC -3' (SEQ ID No. 22) and

ADRBR-R6: 5'- AAATCTGGGCTCCGGCAGTAGATAAG -3' (SEQ ID No. 23).

These two fragments were amplified by means of 'AmpliTaq gold kits' by Perkin Elmer. In these two fragments the temperature profile was as follows: 94°C 10 min.; 35 cycles: 94°C 30 sec., 56°C [fragment V] or 58°C [fragment VI] 30 sec., 72°C 1 min. and finally 72°C 10 min.

Sequencing was carried out by means of the 'thermo sequenase cycle sequencing kit' by Amersham. The PCR primers described above were used as sequencing primers. ADRBR-F1, ADRBR-F3, ADRBR-F5 and ADRBR-R7. Fragments I, III, V and VII were inserted into the two sequencing pools. Yet, pool 3 contained the sequencing primers ADRBR-F2, F4, F6 and F8; pool 4 contained the sequencing primers ADRBR-R2, R4, R6 and R8. Fragments II, IV, VI, VII were inserted into these two pools.

All PCR and sequencing reactions were carried out in a PTC 225 cycler of MJ Research.

The products of the sequencing reaction were separated on a 100 μ m thick acryl amide gel (5% acryl amide, 7 M urea) and under standard DTE conditions (see Richterich and Church, 1993) transferred to a biodyne A membrane (Pall). Then, the membrane was hybridized with 32 P-marked oligonucleotides and the individual sequence leaders were detected with the aid of a phosphor fluorimager (Storm 860, Molecular Dynamics). --